Immunohematology Case Studies 2017 -6

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Clinical History

- A 5 year old boy was admitted to Tehran Children's Hospital due to abdominal pain
- He looked very weak and pale skin with moderate anemia and hemoglobin (Hb) value of 8.1 g/dl
- He had no history of blood transfusion previously
- Four months earlier, he was treated for gingiva infection with Cephalexin but currently he was not under any medication
- His peripheral blood smear showed moderate anemia with slight spherocytosis, schistocytes, moderate microcytes and macrocytes and reticulocytosis

Clinical History

Hematology results

Index	Admission day	10 days later	27 days later	Reference Range
WBC	10300	7600	8100	4400-11000
RBC	2.49	3.40	4.99	3.9-5.3
Hg	8.1	9.7	12.9	11.5-13.5
Hct	24.2	30.4	39.7	34-40
MCV	97.2	89.4	83.6	75-87
MCH	32.5	28.5	25.6	24-30
MCHC	33.5	31.9	30.9	31-37
Reticulocyte	14.3	11.4	0.9	0.5-2.5%
Plt	433	456	378	150-450

Clinical History

- No further laboratory report of anemia was available
- Child became a candidate for splenectomy due to enlarged spleen
- Before surgery because of child's anemic condition 2 units of RBCs were requested

Serologic History

- Hospital blood bank performed routine antibody screening test and reported a positive test results
- Patient's blood sample was referred to IRL for further antibody identification workup and 2 units of antigen negative compatible RBCs was requested
- Due to the limited resources including reagents and personnel, only antibody screening tests are performed as part of pre-transfusion testing by hospital blood banks in Iran
- Currently, main IRL located at the IBTO's headquarter and other 31 special serology laboratories (one in each province) in Iran take the responsibility of antibody ID testing and providing antigen negative and compatible RBC units for the blood bank hospitals

Current Sample Presentation Data at Immunohematology Reference laboratory

ABO/Rh(D): A Rh positive

Antibody Screen Method: IAT using Column Agglutination Technology (CAT) polyspecific (MTC, INVITROGEL, Germany)

Antibody screen Results: Cell 1 (R_1R_1) & Cell 3 (rr) negative but Cell 2 (R_2R_2) positive (reaction grade 2+)

Antibody Identification Method : LISS tube IAT untreated cells

Antibody Identification Results: Only cells with E antigen were reacting in AHG phase (Anti-IgG,C3d) (Bio-Rad laboratories, USA) and negative in immediate spin and 37C phases

Locally produced commercial ID panel

Rh-hr	Cell No	D	C	c	E	e	C^w	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	N	S	s	IS	37° C	Anti-IgG,C3d	CC
rr	**1**	0	0	+	0	+	+	+	+	+	+	+	+	0	+	+	+	+	+	0	0	0	**0**	✓
r'r'	**2**	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	0	0	**0**	✓
r'r	**3**	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	0	0	**0**	✓
r"r	**4**	0	0	+	+	+	0	0	+	0	w	+	0	0	+	+	+	0	+	+	0	0	**2+**	
rr	**5**	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	0	+	0	0	**0**	✓
R2R2	**6**	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	0	0	**2+**	
R2R2	**7**	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	+	+	+	0	0	**2+**	
R1R1	**8**	+	+	0	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	0	0	**0**	✓
RzR2	**9**	+	+	+	+	0	0	0	+	+	0	+	+	+	0	0	+	0	+	0	0	0	**2+**	
R1R1	**10**	+	+	0	0	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	0	0	**0**	✓
R1R1	**11**	+	+	0	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	0	0	**0**	✓
Patient Cell	**12**	0	0	+	0	+	0	0	+	+	+	+	0	0	+	0	0	+	0	+	0	0	**3+**	

IS: Immediate Spin
CC: Check Cells

Challenge with the Current Presentation

- The five year old boy was never transfused with RBCs previously
- Reacting cell number 2 in antibody screening test & ID panel cells numbers 4,6,7,9 were the only RBCs expressing E antigen and all non-reacting cells were negative for E antigen
- First evaluation of the ID panel indicates the presence of anti-E in patient's plasma, but patient had no history of blood transfusion
- A positive auto control test (3+ reactivity) at AHG phase was a clue to possible presence of a warm auto immune anti-E-like in the patient's serum

Challenge with the Current Presentation

- Challenge is to use antibody characteristics and Immunohematology techniques to evaluate and discriminate his anti-E as being an anti-E-like, mimicking an alloantibody vs true alloantibody to provide prompt, best patient blood management
- Next challenge would be to determine if the antibody is clinically significant
- Following procedures were considered:
  - Direct Antiglobulin Test (DAT)
  - Extended phenotype of the patient's blood cell antigens using serologic method and if possible with molecular method to rule out presence of variant RHCE alleles
  - Acid elution procedure of the patient's RBCs
  - Alloadsorption of the patient's plasma sample
  - Titration of antibody

Further Work

- Results of initial direct antiglobulin test (DAT) using test tube method

PS1	PS2	Anti-IgG	Anti-C3d	Control
3+	3+	2+	0✓	0

- Mimicking alloantibodies most frequently are IgG

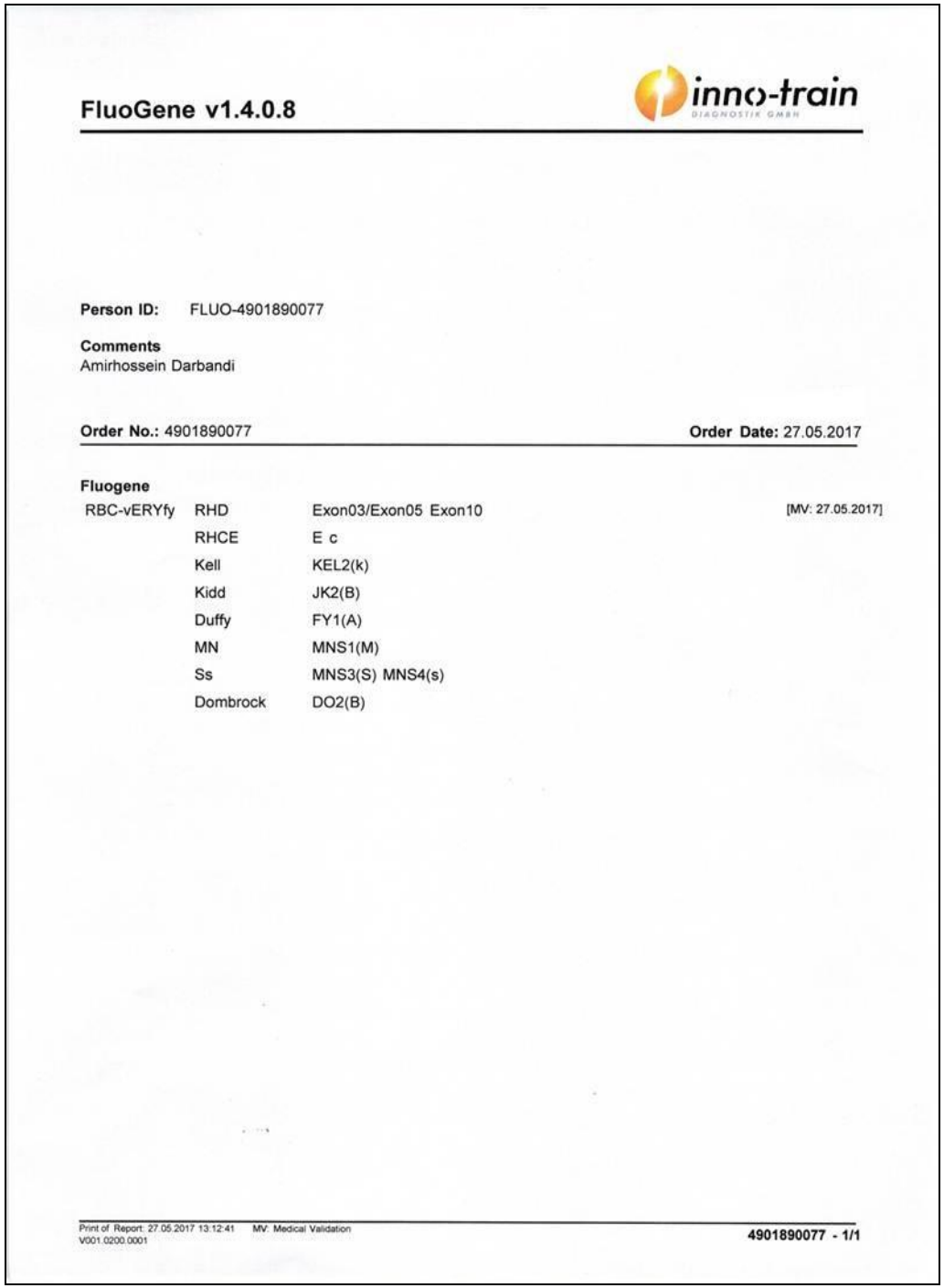
Further Work

- Patient's extended phenotype /genotype

Method	D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	M	N	S	s	Doa	Dob
Serology	+	0	+	+	0	0	+	+	0	0	+	+	0	+	+	NT	NT
Molecular	+	0	+	+	0	0	+	+	0	0	+	+	0	+	+	0	+

NT: Not Tested

No discrepancy between phenotyping and genotyping results was observed, confirming patient as E+



FluoGene v1.4.0.8

inno-train DIAGNOSTIK GMBH

Person ID: FLUO-4901890077

Comments
Amirhossein Darbandi

Order No.: 4901890077 **Order Date:** 27.05.2017

Fluogene

RBC-vERYfy	RHD	Exon03/Exon05 Exon10	[MV: 27.05.2017]
	RHCE	E c	
	Kell	KEL2(k)	
	Kidd	JK2(B)	
	Duffy	FY1(A)	
	MN	MNS1(M)	
	Ss	MNS3(S) MNS4(s)	
	Dombrock	DO2(B)	

Print of Report: 27.05.2017 13:12:41 MV: Medical Validation
V001.0200.0001

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ISBT

Further Work

Antigen Status

- Serology and molecular testing confirmed the patient's phenotype to be R_2R_2 thus E antigen is present on the patient's RBCs
- Molecular results did not show clue of any *RHCE* variants, further molecular testing was not performed

Acid elution procedure

- An acid elution of the patient's RBCs was performed with a rapid acid elution kit (Lorne laboratories, UK)
- Anti-E was observed by reactivity of patient's RBCs eluate using the same antibody ID panel (showing 1+ to 2+ reactions with cells expressing E antigens and no reactions with cell negative for E antigens)

Further Work

Adsorption procedure

- An alloadsorption of the plasma was performed with enzyme (papain) treated cells. R_2R_2 (DcE/DcE) or rr (dce/dce) multiple aliquots (1ml) of cells were used .One ml plasma was added, mixed and incubated at 37C for 45 minutes (repeated X2)
- A commercial anti-E reagent was used and tested identically as a control
- The adsorbed plasma was tested with several E antigen positive and E antigen negative cells from ID panel using LISS as enhancement medium

Further Work

Results of a selected cell panel repeated with allogenic adsorbed plasma and control anti-E reagent

Rh-hr	Cell No	D	C	c	E	e	C^w	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	N	S	s	Patient plasma adsorbed with R2R2	Patient plasma adsorbed with rr	Control plasma adsorbed with R2R2	Control plasma did not adsorb with rr
rr	**1**	0	0	+	0	+	+	+	+	+	+	+	+	0	+	+	+	+	+	0	0	0	0	0
r'r'	**2**	0	+	0	0	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	0	0	0	0
R2R2	**3**	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	0	0	0	3+
R2R2	**4**	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	+	+	+	0	0	0	3+
R1R1	**5**	+	+	0	0	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	0	0	0	0
R1R1	**6**	+	+	0	0	+	0	0	+	+	0	0	+	0	+	0	+	+	+	+	0	0	0	0

Updated Clinical Information

- IRL discussed the presence of anti-E-like antibody in the patient's plasma with the hematology specialist treating him. He decided not to transfuse RBC units but treat the boy with anti-inflammatory corticosteroids (prednisolone)

Prednisolone dose prescribed	Doses for 28 weeks
75 mg	3 times daily
50 mg	2 times daily
25 mg	1 time daily
12.5 mg	Every other day

Updated Clinical Information

•Chronological changes in patient's hematological test during 28 weeks

Date	Hg g/dl	Antibody screen results	Anti-IgG+C3d	Anti-IgG	Anti-C3d	Auto Control
Admission day	8.1	anti-E-like antibody	Positive	2+	0✓	3+
4 weeks later	12.9	Negative	Positive	1+	0✓	2+
10 weeks later	14.0	Negative	Positive	Weak+	Weak+	1+
24 weeks later	12.0	Negative	0✓	0✓	0✓	Weak+
28 weeks later	13.1	Negative	0✓	0✓	0✓	0✓

- Approximately 70 percent of blood donors are negative for E antigen in Iran. It was not difficult to prepare E negative RBC units for the patient . During the course of treatment the child's hemoglobin level was within the normal range and he did not need any blood transfusion
- RBC hemolysis was prevented and surgery for splenectomy was cancelled

Conclusions

- By performing relevant immunohematology procedures, it was concluded that the antibody present in this anemic child with no history of blood transfusion was an antibody of E-like specificity
- No alloantibody specificity to any other RBC antigens was observed
- Obtaining patient's clinical history was essential for clues in antibody identification studies. Patient's history was especially helpful since the results on autocontrol test and DAT were positive
- RBCs transfusion and splenectomy surgery were cancelled and it was decided that the patient should be treated with prednisolone

Summary of Case Challenges

- Mimicking alloantibodies may interfere with timely release of compatible blood products or making a sound clinical decision caused by laboratory confusion and clinical team
- Laboratory staff awareness of patient's clinical condition and accessibility to that information is important for efficient laboratory workup
- Children with WAIHA only rarely have an underlying chronic disease, and the vast majority of pediatric cases follow infection episodes or may be idiopathic, and transient

Lessons Learned by the Case

- The diagnosis of WAIHA requires corroborating evidence of clinical signs, symptoms or laboratory findings indicative of immune hemolysis

- Close collaboration and counselling between the Immunohematology Reference Laboratory and the treating physician is essential for fast, successful and more efficient patient blood management, and prevention of unnecessary blood transfusion

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